

This listing of claims will replace all prior versions, and listings, of claims in the application:

In the Claims

Claim 1 (currently amended): A method of producing a mammalian cell for packaging of a recombinant AAV (rAAV) vector, said method comprising the steps of:

~~(a) providing a mammalian cell which comprises a stably integrated AAV cap gene operably linked to a promoter, and a stably integrated AAV rep gene operably linked to a helper virus inducible heterologous promoter, wherein the AAV cap gene and the AAV rep gene are stably integrated into the mammalian cell's genome, wherein p5 promoter function has been replaced by the helper virus inducible heterologous promoter; and wherein said mammalian cell is prepared by introducing a single plasmid comprising AAV rep and AAV cap arranged as in the AAV genome into the mammalian cell;~~

(b) (a) replicating the a mammalian cell of step (a) to produce a population of cells; and wherein the mammalian cell comprises a stably integrated AAV cap gene operably linked to AAV p40 promoter, and a stably integrated AAV rep gene operably linked to a helper virus-inducible heterologous promoter, wherein the AAV cap gene and the AAV rep gene are stably integrated into the mammalian cell's genome, wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter; and wherein said mammalian cell is prepared by introducing a single plasmid comprising AAV rep and AAV cap arranged as in the AAV genome into the mammalian cell;

(c) (b) introducing a helper virus to the population of cells of step (b) (a); and

(d) (c) wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

Claim 2 (previously presented): The method according to claim 1, wherein said helper virus is an adenovirus.

Claim 3 (previously presented): The method according to claim 1, wherein said packaging cell grows at least one half as rapidly as parental-type cells that do not contain an AAV rep gene, and wherein said packaging cell when used to package rAAV vectors produces at least 100 rAAV particles/cell.

Claim 4 (cancelled)

Claim 5 (previously presented): The method according to any of claims 1-3, wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

Claim 6 (previously presented): A cell produced by the method of claim 1, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

Claim 7 (previously presented): A cell produced by the method of claim 3, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

Claim 8 (cancelled)

Claim 9 (previously presented): A cell produced by the method of claim 5, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

Claim 10 (currently amended): A mammalian cell for packaging of a recombinant AAV (rAAV) vector, said cell comprising a stably integrated cap gene operably linked to a AAV p40 promoter, and a stably integrated rep gene operably linked to a helper virus-inducible

heterologous promoter; wherein the cap gene and the rep gene are stably integrated into the mammalian cell's genome; wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter; wherein said cell exhibits helper-virus-inducible expression of said stably integrated AAV rep gene; and wherein said mammalian cell is prepared by introducing a single plasmid comprising rep and cap arranged as in the AAV genome into the mammalian cell.

Claim 11 (previously presented): The AAV packaging cell of claim 10, wherein said helper-virus-inducible expression of said stably integrated AAV rep gene is inducible by adenovirus.

Claim 12 (previously presented): The AAV packaging cell of claim 10, wherein said packaging cell grows at least one half as rapidly as parental-type cells that do not contain an AAV rep gene, and wherein said packaging cell when used to package rAAV vectors produces at least 100 rAAV particles/cell.

Claim 13 (cancelled)

Claim 14 (previously presented): The AAV packaging cell of any of claims 10-12, wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

Claim 15 (previously presented): The AAV packaging cell of claim 10, further comprising a stably integrated recombinant AAV (rAAV) vector, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter.

Claim 16 (currently amended): A method of packaging a recombinant AAV vector, comprising the ~~steps~~ step of:

~~(a) introducing a recombinant AAV (rAAV) vector into the AAV packaging cell of claim 10, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter;~~

~~(b) introducing a helper virus; and~~

~~(c) incubating the an AAV packaging cell of claim 10 under conditions suitable for replication and packaging of AAV such that said a recombinant AAV(rAAV) vector is packaged; wherein the AAV packaging cell comprises the rAAV vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter; and wherein the AAV packaging cell comprises a helper virus.~~

Claim 17 (currently amended) A method of packaging a recombinant AAV vector, comprising the ~~steps~~ step of:

~~(a) introducing a helper virus into an AAV packaging cell of claim 15 which comprises a stably integrated rAAV vector comprising a polynucleotide of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter; and~~

~~(b) incubating the an AAV packaging cell of claim 15 under conditions suitable for replication and packaging of AAV such that said a stably integrated rAAV vector is packaged; wherein the AAV packaging cell comprises a helper virus; and wherein the AAV packaging cell comprises the stably integrated rAAV vector comprising a polynucleotide of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter.~~

Claims 18-20 (cancelled)

Claim 21 (previously presented): A method of determining the infectious titer of an rAAV vector preparation, comprising the steps of:

(a) introducing a helper virus and serial dilutions of the rAAV vector preparation to AAV packaging cells of claim 10;

(b) incubating the cells under conditions suitable for replication of AAV; and

(c) determining the amount of replicated rAAV vector relative to an rAAV preparation of known titer.

Claim 22 (previously presented): The method of claim 1, further comprising the step of selecting a cell exhibiting helper-virus-inducible expression of said stably integrated AAV rep gene.

Claim 23 (cancelled)

Claim 24 (currently amended): A method of packaging a recombinant AAV vector, comprising the ~~steps~~ step of:

~~(a) preparing an AAV packaging cell according to the method of claim 1, wherein the AAV packaging cell exhibits helper virus-inducible expression of the stably integrated AAV rep gene;~~

~~(b) introducing a recombinant AAV (rAAV) vector into the AAV packaging cell, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter; and~~

~~(c) incubating the an AAV packaging cell prepared according to the method of claim 1 under conditions suitable for replication and packaging of AAV such that said a~~

recombinant AAV (rAAV) vector is packaged; wherein the AAV packaging cell comprises the rAAV vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter; and wherein the AAV packaging cell exhibits helper virus-inducible expression of the stably integrated AAV rep gene.

Claim 25 (currently amended): A method of producing a mammalian cell for packaging of a recombinant AAV (rAAV) vector, said method comprising the step of:

(a) introducing a single plasmid comprising AAV rep and AAV cap arranged as in the AAV genome into a mammalian cell, wherein the AAV cap gene is operably linked to a AAV p40 promoter and the AAV rep gene is operably linked to a helper virus-inducible heterologous promoter, wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter; wherein the plasmid becomes stably integrated into the mammalian cell's genome; and wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

Claim 26 (previously presented): The method according to claim 25, wherein said helper virus is an adenovirus.

Claim 27 (previously presented): The method according to claim 25, wherein said packaging cell grows at least one half as rapidly as parental-type cells that do not contain an AAV rep gene, and wherein said packaging cell when used to package rAAV vectors produces at least 100 rAAV particles/cell.

Claim 28 (previously presented): The method according to any of claims 25-27, wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

Claim 29 (previously presented): A cell produced by the method of claim 27, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

Claim 30 (previously presented): A cell produced by the method of claim 25, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

Claim 31 (previously presented): A cell produced by the method of claim 28, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

Claim 32 (previously presented): A cell produced by the method of claim 25, and progeny thereof, wherein said cell further comprises a stably integrated recombinant AAV (rAAV) vector, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter.

Claim 33 (currently amended): A method of packaging a recombinant AAV vector, comprising the ~~steps~~ step of:

~~(a) introducing a recombinant AAV (rAAV) vector into the AAV packaging cell of claim 29, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter;~~

~~(b) introducing helper virus; and~~

~~(c) incubating the an AAV packaging cell of claim 29 under conditions suitable for replication and packaging of AAV such that a recombinant AAV (rAAV) vector is packaged;~~

wherein the AAV packaging cell comprises the rAAV vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter; and wherein the AAV packaging cell comprises a helper virus.

Claim 34 (currently amended): A method of packaging a recombinant AAV vector, comprising the ~~steps~~ step of:

- (a) ~~introducing a helper virus into an AAV packaging cell of claim 32; and~~
- (b) ~~incubating the an AAV packaging cell of claim 32 under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged; wherein the AAV packaging cell comprises a helper virus.~~

Claims 35-37 (cancelled)

Claim 38 (currently amended): A method of packaging a recombinant AAV vector, comprising the ~~steps~~ step of:

- (a) ~~preparing an AAV packaging cell according to the method of claim 25, wherein the AAV packaging cell exhibits helper virus inducible expression of the stably integrated AAV rep gene;~~
- (b) ~~introducing a recombinant AAV (rAAV) vector into the AAV packaging cell, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter;~~
- (c) ~~introducing a helper virus; and~~
- (d) incubating the an AAV packaging cell prepared according to the method of claim 25 under conditions suitable for replication and packaging of AAV such that said a recombinant AAV (rAAV) vector is packaged; wherein the AAV packaging cell exhibits helper virus-inducible expression of the stably integrated AAV rep gene; wherein the AAV packaging

cell comprises the rAAV vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter; and wherein the AAV packaging cell comprises a helper virus.

Claim 39 (previously presented): The method of claim 33, wherein said helper virus is an adenovirus.

Claim 40 (previously presented): The method of claim 34, wherein said helper virus is an adenovirus.

Claim 41 (previously presented): The method of claim 38, wherein said helper virus is an adenovirus.